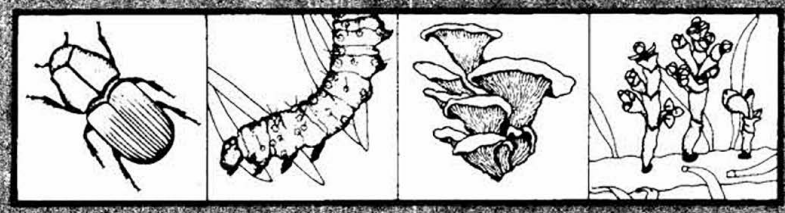
Forest Pest Management



Report 91-7

3450
August 1991

DWARFING OF BAREROOT WESTERN LARCH SEEDLINGS USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

R. L. James[1], Plant Pathologist; W. Antrobius, Entomologist; C. J. Gilligan, Biological Technician

ABSTRACT

Bareroot western larch seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho were smaller in rows close to irrigation structures which remained in the field during soil fumigation. Reduced seedling height was most pronounced during the second growing season and was related to higher soil populations and subsequent root infection by *Pythium ultimum* and *P. debaryanum*. Although *Fusarium* spp. were commonly detected within soil and on roots of seedlings, only levels of root infection by *Pythium* were significantly correlated with distance from irrigation lines. Seedling heights, but not density, were also significantly correlated with distance from irrigation lines. Removing irrigation structures prior to soil fumigation and providing adequate soil drainage should help reduce future impact of *Pythium* root diseases at the nursery.

INTRODUCTION

During the 1989 growing season at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, 2+0 bareroot western larch (*Larix occidentalis* Nutt.) seedlings were consistently smaller in the first two rows adjacent to irrigation structures (figure 1). Affected nursery fields had been fumigated with a granular formulation of dazomet (Basamid®) during the early fall, preceding sowing of western larch the following spring. However, above-ground irrigation structures remained in the field during the fumigation treatment. Therefore, soil underneath and adjacent to irrigation structures probably escaped fumigation.

---

[1] Stationed in Coeur d'Alene, Idaho

United States Department of Agriculture | Forest Service | **Northern Region** | P.O. Box 7669 Missoula, Montana 59807

Figure 1.--Bareroot western larch seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho with reduced height adjacent to irrigation lines.

During the early part of the first growing season, larch seedling establishment and height were about the same regardless of proximity to irrigation structures. However, by the end of the first growing, differential seedling height became quite evident. Some seedlings nearest irrigation structures also had slightly chlorotic foliage when compared to seedlings located farther away. Seedling density at varying distances from irrigation structures appeared similar.

Growers were concerned that less-effective soil fumigation near the irrigation structures resulted in increased seedling infection by pathogenic fungi residing in soil near the structures. Therefore, we investigated the relationships between seedling dwarfing and amounts of soil colonization and seedling infection by common root-pathogenic fungi.

MATERIALS AND METHODS

Twelve field plots were established within beds of 2+0 bareroot western larch seedlings during spring 1989. Plots were located adjacent to four rows of irrigation structures and delimited with wooden stakes at their four corners. Plots were located either directly north or south of structures. Each plot contained seven rows of seedlings, designated numerically with row 1 being closest to irrigation lines.

Soil samples were collected from four randomly selected plots. Soil from all plots was not sampled because of the high costs involved in assays. Samples were collected in May and September 1989. Within each sampled plot, five soil samples were collected sequentially at increasing distances from irrigation structures. The first sample was obtained adjacent to the structure. Sample 2 was collected between the structure and first row of seedlings, sample 3 from between seedling rows 1 and 2, sample 4 between seedling rows 3 and 4, and sample 5 between seedling rows 5 and 6. Each sample consisted of a 23 mm core of soil taken to a depth of 15 cm. Soil from each location was placed in a paper bag, thoroughly mixed, and kept refrigerated until analyzed.

Standard soil dilution techniques were used to assay for two groups of potential soil pathogens: *Fusarium* and *Pythium* spp. Soil was initially sieved to remove rocks, pieces of organic matter, and soil aggregations. For analysis of pathogen populations, field-moist soil was used, but fungal populations were reported on an oven-dry weight basis. From each soil sample, a 5 g subsample was dried at about 100°C for a least 24 hours until sampled weight had stabilized (all excess moisture removed) to calculate oven-dry weight. For assay of *Fusarium* populations, 0.05 g of moist soil was weighed from each sample, combined with 100 ml of 0.3 percent water agar (WA) and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium for *Fusarium* (Komada 1975), and spread uniformly over the agar surface. Plates were incubated at about 24°C under diurnal cycles of cool, fluorescent light for 5-7 days. *Fusarium* colonies were determined by their morphology on the selective medium and colony-forming units (cfu) per g of soil calulated. Similar procedures were used for assay of *Pythium* populations except 5.0 g of moist soil were initially introduced into WA and the solution placed on a selective *Pythium* agar medium consisting of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene. Plates with soil were incubated at about 24°C for 3 days in the dark. After incubation, excess soil was carefully washed from the surface of plates and number of *Pythium* colonies determined. Colonies were identified by their diameter after 3 days (15-20 mm), their feathery margin, and whether they grew within, rather than superficially on, the surface of agar; cfu/g of soil were then calculated.

In fall 1989, just before lifting, the number of seedlings per row were counted within plots. Heights from the ground to the tip of the terminal bud were measured for each seedling. One seedling without disease symptoms (foliage appeared healthy) in rows 1, 2, 3 and 7 was randomly selected, carefully excavated, and sampled for root colonization by potentially pathogenic fungi. Roots of sampled seedlings were washed thoroughly under tap water to remove pieces of soil. Randomly selected roots were excised into pieces 2-3 cm in length, which were surface sterilized in 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite), rinsed in sterile water, and placed on the two selective media used for soil dilutions. Plates were incubated as described above with selected isolates transferred to potato dextrose agar for identification.

Identification of *Fusarium* spp. was facilitated by growing isolates on carnation leaf agar which stimulated sporodochial production and uniformity of macroconidial morphology (Fisher and others 1982) and on a medium of low water potential which allowed microconidial chains to form for those taxa which produce them (Nirenberg 1981). The taxonomic descriptions of Nelson and others (1983) was used to classify *Fusarium* spp.

Isolates of *Pythium* were classified using the monographs of Middleton (1943) and Waterhouse (1968); those of *Cylindrocarpon* were identified from descriptions by Booth (1966).

Data were analyzed using standard analyses of variance (ANOVA). Statistical differences among means were located using Fisher's Least Significant Difference Test. The Lilliefors Test was used to test for normality, and Bartlett's Test was used to test for homogeneity in the ANOVA models. A level of significance with P=0.05 was used for all tests.

RESULTS AND DISCUSSION

Seedling Density and Height

Number of seedlings within plots by rows at increasing distances from irrigation structures is summarized in table 1. Although some statistical differences (P=0.05) existed among plots and one of the rows, the differences were not consistently associated with distance from irrigation structures. There were significantly more seedlings in row 4 than in other rows, but all other rows had statistically similar numbers of seedlings. Conversely, seedling heights in the two rows nearest the structures were significantly shorter (P=0.05) than those in other rows (table 2). Heights progressively increased with greater distance from irrigation lines, with those in row 7 (farthest from lines) being the tallest. Some significant differences in height were also recorded among the 12 plots, possibly indicating non-uniformity of growing areas or seedlot differences.

Table 1.--Number of bareroot western larch seedlings by row from irrigation lines and plots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Number of Seedlings Row[2]									
Plot[1]	1	2	3	4	5	6	7	Total	Avg.[3]	SD[4]
1	17	13	10	18	9	5	9	81	11.6	4.7
2	11	8	7	29	17	7	8	78	11.1	5.3
3	11	8	11	6	8	7	2	53	7.6	3.1
4	12	13	13	13	11	17	5	84	12.0	3.6
5	9	8	7	18	15	17	8	82	11.7	4.7
6	14	11	6	28	9	14	20	102	14.6	6.4
7	15	8	7	9	13	11	11	74	10.6	2.8
8	14	9	6	12	6	10	12	69	9.9	3.1
9	3	5	6	6	3	7	7	37	5.3*	1.7
10	7	17	22	16	8	8	17	95	13.6*	5.8
11	10	7	4	10	17	6	5	59	8.4	4.4
12	12	11	10	12	8	8	4	65	9.3	2.8
Total	135	118	109	168	124	117	108	897	---	---
Avg.[3]	11.2	9.8	9.1	14.0*	10.3	9.7	9.0	---	---	---
SD[4]	3.8	3.3	4.8	6.4	4.4	4.2	5.2	---	---	---

[1]ANOVA indicated that there were significant differences among plots (F = 2.703; P=0.006).
[2]Rows were sequentially farther from the irrigation structure (row 1 was closest; row 7 was farthest away). Analysis of variance indicated that there were significant differences among rows (F = 2.217; P=0.052).
[3]Means followed by an asterisk are sigificantly different (P=0.05) than the combined means of all other plots or rows using Fisher's Least Significance Difference Test.
[4]Standard deviation.

Table 2.--Average heights (mm) of bareroot western larch seedlings by row from irrigation structures and sample plots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Average Seedling Height (cm) Row[2]								
Plot[1]	1	2	3	4	5	6	7	Avg.[3]	SD[4]
1	8.8	15.4	19.1	18.2	15 6	21 2	23 3	16.3	7.2
2	6.5	11.4	13.0	15.6	17.2	15.6	23.8	14.9	6.3
3	7.6	7.5	9.0	15.1	12.2	21.3	38.8	13 7	6.3
4	12 2	10.7	14.3	14.0	15.1	22.0	20.8	15.5	7.9
5	8.9	11.5	11.4	8.7	14.0	14.6	20.4	12.6*	6.0
6	10.7	19.0	18.5	14.9	23.	21 5	21.1	17.9	9.7
7	14.8	20.9	29.5	25.9	20.5	12.4	18.8	19.4*	9.0
8	7.5	7.0	19.2	12.1	15.2	9.4	17.1	115.*	7.2
9	15.4	17.5	18.3	25.5	35.1	28.2	26.1	23.8*	11.1
10	9.6	12.9	15.1	20.2	16.8	15.7	20.3	16.3	6.2
11	7.0	10.3	18.2	17.0	17	28.6	24.9	16.3	8.9
12	14.0	13.7	15.2	23.1	22.1	12.4	18.3	16.9	7.6
Avg.[3]	10.1*	13.1*	16.0	16.6	18.1	18.1	21.6*	---	---
SD[4]	48.4	64.9	71.9	79.8	81.7	92.4	95.3	---	---

[1]ANOVA indicated that there were significant differences among plots (F = 9.571; P=0.005).

[2]Rows were sequentially farther from the irrigation structure (row 1 was closest; row 7 was farthest away). ANOVA indicated that there were significant differences among rows (F = 2.217; P=0.052).

[3]Means followed by an asterisk are sigificantly different (P=0.05) than the combined means of all other plots or rows using Fisher's Least Significance Difference Test.

[4]Standard deviation.

Soil Pathogen Populations

Soil populations of *Fusarium* were statistically similar for all plots and sample locations for both sample times (table 3). Fall populations generally exceeded those in spring, although much of this difference was due to a very high population detected in the first sample location of plot 3 (table 3). This high population was unusual and produced a large average population for the first sample location (closest to irrigation lines) in the fall sample. However, due to extreme variability of detected populations, differences were not statistically significant. In a previous study at the Coeur d'Alene Nursery (James and others 1990), slightly higher *Fusarium* populations were detected in the fall rather than spring in some fields with the reverse being true in others. *Fusarium* spp. usually exist in the soil as chlamydospores which tend to decrease in viability with time away from susceptible hosts (Park 1959; Price 1984). These fungi are most active during warm, dry conditions (Bloomberg 1973; Tint 1945) and higher detectable populations are expected in the fall. However, overall *Fusarium* populations in the soil at the Coeur d'Alene Nursery are quite low due to a recurring program of soil fumigation before each seedling crop is sown. In the past, populations have increased slightly before fumigation restored them to very low levels.

Table 3.--Soil populations of *Fusarium* spp. assayed by soil dilution within 2+0 western larch fields at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Colony-Forming Units/g of Soil Plots				
Sample Location[1]	1	2	3	4	Avg.[2]
	Sample Date: 6/89				
1	0	74	0	295	92
2	0	0	148	295	111
3	0	0	0	0	0
4	0	74	148	0	55
5	0	74	221	0	59
Avg.[3]	0	44	103	118	65
	Sample Date: 9/89				
1	67	0	2939	295	92
2	67	67	134	467	184
3	67	134	134	0	84
4	67	0	67	0	33
5	267	0	67	0	84
Avg.[3]	107	40	668	93	227

[1]Sample locations within plots = sample 1 adjacent to irrigation structure; sample 2 between the irrigation structure and first row of seedlings; sample 3 between seedling rows 1 and 3; sample 4 between seedling rows 3 and 4; sample 5 between seedling rows 5 and 6.

[2]ANOVA indicated no significant differences in *Fusarium* populations among the sample locations (6/89: F = 0.799; P=0.549; 9/89: F = 0.813; P=0.541).

[3]ANOVA indicated no significatn differences in *Fusarium* populations maong the plots (6/89: F = 1.643; P=0.232; 9/89: F = 0.998; P=0.427).

In contrast with *Fusarium*, soil populations of *Pythium* spp. were consistently higher nearer irrigation lines (table 4). Populations from the first sample location (nearest the lines) were significantly higher (P=0.05) than those from locations farther away from structures. Spring populations of these fungi were higher than those detected in the fall. Since *Pythium* spp. are favored by wet soil conditions (Vaartaja and Bumbieris 1964), they are expected to proliferate in the spring when natural rainfall is prevalent at the Coeur d'Alene Nursery compared to summer and fall when rainfall is generally less. Different *Pythium* populations in spring and fall were previously detected at the nursery (James and others 1990), but consistent relationships in levels at different times of the year varied among sample locations. Some portions of the nursery, particularly those with poor drainage, may have recurring problems associated with high *Pythium* populations (James 1982; James and Gilligan 1986a). Populations detected in the present investigation are similar to those previously estimated in other portions of the nursery (James and Gilligan 1986b; James and others 1990). Generally, *Pythium* populations exceeding about 100 cfu/g are considered high enough to cause concern (Campbell and Kelpsas 1988; Vaartaja and Bumbieris 1964). Although populations exceeding this threshold level sometimes result in damage to seedling stock, it is usually difficult to predict crop damage from soil populations. Existing assay techniques fail to

differentiate between pathogenic and saprophytic fungal strains, and seedling damage is often closely associated with environmental conditions, particular soil moisture (Edmonds and Heather 1973).

Table 4.–Soil populations of *Pythium* spp. assayed by soil dilution within 2+0 western larch fields at the Forest Service Nursery, Coeur d'Alene, Idaho.

	Colony-Forming Units/g of Soil Plots				
Sample Location[1]	1	2	3	4	Avg.[2]
	Sample Date: 6/89				
1	148	214	184	310	214*
2	214	280	96	251	210*
3	59	103	7	22	48*
4	74	103	7	37	55*
5	15	15	52	59	35*
Avg.[3]	102	143	69	136	112
	Sample Date: 9/89				
1	47	87	27	13	43*
2	27	47	27	7	27
3	7	7	7	13	8
4	7	13	0	0	5
5	7	7	0	0	4*
Avg.[3]	10	32	12	7	17

[1]Sample locations within plots = sample 1 adjacent to irrigation structure; sample 2 between the irrigation structure and first row of seedlings; sample 3 between seedling rows 1 and 3; sample 4 between seedling rows 3 and 4; sample 5 between seedling rows 5 and 6.

[2]ANOVA indicated no significant differences in *Pythium* populations among the sample locations (6/89: F = 13.667; P=0.0005; 9/89: F = 6.328; P=0.006). For each sample date, means followed by an asterisk are signifcantly different (P=0.05) than combined means of all other sample locations using Fisher's Least Significant Difference Test.

[3]ANOVA indicated no significatn differences in *Pythium* populations among the plots (6/89: F = 2.366; P=0.122; 9/89: F = 3.211; P=0.062).

Seedling Root Infection

Roots of most sampled seedlings were colonized by *Fusarium* spp., especially *F. oxysporum* Schlecht. (table 5). Intensity of root colonization, i.e., percent of sampled root pieces colonized, was almost 25 percent. High levels of these fungi were detected on roots even though sampled seedlings lacked above-ground disease symptoms. Although seedlings in the first two sample rows were usually shorter than those in other rows (table 2), they otherwise appeared healthy. Such high levels of *Fusarium* colonization of non-diseased seedlings, although common on container-grown stock at the Coeur d'Alene Nursery (James and Gilligan 1988a), is unusual for bareroot seedlings (James and Gilligan 1988b). Other species of *Fusarium* isolated from roots of

bareroot larch seedlings included *F. acuminatum* Ell. & Ev., *F. proliferatum* (Matsushima) Nirenberg, *F. sambucinum* Fuckel, *F. solani* (Mart.) Appel & Wollen., and *F. tricinctum* (Corda) Sacc. Some of these species may be pathogenic on conifer seedlings (James and Gilligan 1984; James and others 1989). However, many are undoubtedly saprophytic colonizers of host cortical cells or the rhizosphere (Bloomberg 1976).

Table 5.--Isolation of selected fungi from roots of non-diseased 2+0 western larch seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent Seedlings Infected[1] Sample Row[2]				
Fungus	1	2	3	7	Avg.
Fusarium oxysporum	75.0	91.7	66.7	50.0	70.8
Other *Fusarium* spp[3]	66.7	58.3	66.7	50.0	60.4
All *Fusarium*	83.3	100.0	100.0	66.7	87.5
Pythium spp.	50.0	50.0	25.0	25.0	37.5
Other "water molds"[4]	91.7	75.0	91.7	91.7	87.5
Cylindrocarpon spp.	100.0	100.0	100.0	100.0	100.0
Trichoderma spp.	33.3	50.0	50.0	58.3	47.9
Phoma spp.	41.7	33.3	41.7	41.7	39.6
	Fungal Colonization Intensity[5]				
Fusarium oxysporum	20.0	20.8	12.5	6.7	15.0
Other *Fusarium* spp[3]	9.2	8.3	8.3	5.8	7.9
All *Fusarium*	29.2	29.2	20.8	12.5	22.9
Pythium spp.[6]	14.2A	8.3AB	3.3B	3.3B	7.3
Other "water molds"[4]	33.3	28.3	37.5	32.5	32.5
Cylindrocarpon spp.	80.8	76.7	85.0	88.3	82.7
Trichoderma spp.	6.7	7.5	5.0	12.5	7.9
Phoma spp.	7.5	6.73	11.7	5.8	7.9

[1]One seedling randomly sampled in each of four rows within each plot = 48 total seedlings sampled.

[2]Rows designated sequentially from irrigation structures, i.e., row 1 closes to structures, row 7 farthest away.

[3]Includes *F. acuminatum,F. proliferatum, F. sambucinum, F. solani, and F. tricinctum.*

[4]Other fungi which grew readily on V-8 juice selective medium; includes primarily Zygomycetes (*Mortierella* and members of the Mucorales).

[5]Percent of root pieces colonized with appropriate fungus; then pieces sampled per seedling. ANOVA indicated no significant differences (P=0.05) in root colonization intensity for each fungus among the different seedling rows except for *Pythium* spp. (see [6]). The following statistical values apply for comparisons among seedling rows:

Fungus	F. Value	P
Fusarium oxysporum	2.786	0.056
Other *Fusarium* spp.	0.327	0.806
Other "water molds"	1.694	0.788
Cylindrocarpon spp.	1.054	0.382
Trichoderma spp.	0.533	0.663
Phoma spp.	0.698	0.560

[6]Within the *Pythium* spp. row, means followed by the same capital letter are not significantly different (P=0.05) using Fisher's Least Significant Difference Test.

Fusarium oxysporum exists within soil as either pathogenic or saprophytic strains (Armstrong and Armstrong 1975; Park 1959). This species is especially well adapted to colonize cortical tissues on roots of many different types of host plants (Park 1959). However, pathogenic strains of *F. oxysporum* are usually host specific and designated as formae specialis based on this specificity (Armstrong and Armstrong 1975). Unfortunately, saprophytic and pathogenic strains of *F. oxysporum* are morphologically similar (Matuo and Chiba 1966) and genetic (Kuninaga and Yokosawa 1989; Puhalla 1985) or biochemical (Iannelli and others 1982) tests are required to differentiate them. Because of the general lack of disease symptoms on western larch seedlings, we suspect most isolates of *F. oxysporum* obtained from seedling roots in this study were saprophytic.

Cylindrocarpon spp. were isolated from the roots of all sampled seedlings and colonized a high proportion of root systems (table 5). Most isolates were identified as *C. destructans* (Zins.) Scholten. Two other species, *C. didymum* (Hartig) Wollenw. and *C. tenue* Bugn., were infrequently isolated. *Cylindrocarpon* spp. are common colonizers of conifer seedling rhizospheres (James 1988; Matturi and Stenton 1964) and isolates may be pathogenic or saprophytic (Matturi and Stenton 1964). Although capable of causing root decay without eliciting disease symptoms on seedlings (James and Gilligan 1990), isolates of these fungi were encountered at about equal frequency on western larch seedlings at varying distances from irrigation lines in the present evaluation. Therefore, it is unlikely that they were responsible for the decreased seedling heights found near irrigation structures.

Other fungi assayed on roots of western larch seedlings included *Trichoderma* and *Phoma* spp. (table 5). *Trichoderma* spp. are common soil inhabitants which may be antagonistic toward or competitive with soil-borne pathogens (Papavizas 1985), and *Phoma* spp. are mostly saprophytic but capable of causing disease of conifer seedlings under conducive environmental conditions (James and Hamm 1985). Both these groups of fungi were isolated at about equal frequency from seedlings growing at varying distances from irrigation lines. We suspect that root colonization by these two groups of fungi had no impact of seedling height.

Pythium spp. were isolated from seedling roots at lower levels than *Fusarium* (table 5), despite their greater occurrence in the soil (table 4). Two species of *Pythium* were consistently isolated: *P. ultimum* Trow. and *P. debaryanum* Hesse. Both species are morphologically similar and are best separated on the basis of antheridia, which are usually single and monoclinous in isolates of *P. ultimum* and multiple in *P. debaryanum* (Middleton 1943). Both species occur on many different hosts and soil types (Middleton 1943; Waterhouse 1968) and are often associated with conifer seedling diseases (Hendrix and Campbell 1968; James 1982; Vaartaja 1967). Significantly higher rates of root colonization (P=0.05) were detected on seedlings closer to irrigation structures (table 5). Associations between root infection by *Pythium* spp. and occurrence of foliar chlorosis on seedlings near irrigation lines were previously shown for Engelmann spruce transplants at the Coeur d'Alene Nursery (James 1987). Therefore, we conclude that infection by these fungi was probably the most important factor causing reduced height and vigor of western larch seedlings near irrigation structures.

Besides *Pythium* spp., several other fungi were isolated from seedling roots on the selective V-8 juice agar medium. Although designated as "other water molds" in table 5, these fungi were actually members of the Zygomycetes, including several *Mortierella* spp., *Rhizopus* sp., and *Mucor* sp. *Mortierella* spp. are common soil inhabitants (Domsch and others 1980) and especially found in forest soils (Hayes 1965; Sewell and Brown 1959).

They were likely detected as rhizosphere colonizers in the present investigation, even though care was taken to surface sterilize roots prior to analysis. These fungi are not known to cause disease of conifer seedlings, although their association with and growth on conifer seed has previously caused concern to growers (James 1991).

Pythium - Mortierella Growth Comparisons

One of the most reliable criteria for quickly delineating *Pythium* spp. from *Mortierella* spp. on selective V-8 juice agar medium is growth rate (James and others 1990). However, both groups contain species and strains which grow faster than others, which may obscure growth rate differences. To confirm reliability and stability of growth rate differences, several isolates known to be either *Pythium* or *Mortierella* were compared for their growth on selective V-8 juice agar medium. Results (table 6) indicated consistent growth rate differences between the two groups of fungi, which were statistically significant (P=0.05). The nine isolates of *Pythium* tested included five (#1-5) identified as *P. ultimum* and four (#6-9) which were *P. debaryanum*. The 15 isolates of *Mortierella* included six (#1-3, 12, 13) which were tentatively identified as *M. lana* Linnem., a slower-growing species, and the nine others were probably either *M. alpina* Peyronel or *M. ramanniana* (Moller) Linnem. Even the fastest growing *Mortierella* isolate evaluated was much slower growing than the slowest *Pythium* isolate. Therefore, growth rate on selective V-8 juice agar appears a reliable indicator separating these two groups of important soil-borne fungi.

Table 6.--Comparisons of linear growth at 25°C on V-8 juice agar of *Pythium* spp. and *Mortierella* spp. isolated from roots of 2+0 western larch seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Linear Growth[1]	
Isolates[2]	*Pythium*	*Mortierella*
1	61.0	9.0
2	60.5	7.5
3	58.5	9.0
4	69.0	13.0
5	51.0	18.5
6	45.0	20.0
7	67.5	11.0
8	49.5	20.0
9	51.0	11.0
10	---	16.5
11	---	20.0
12	---	9.5
13	---	5.0
14	---	23.0
15	---	10.0
Avg.[3]	57.0	13.5

[1]Average colony diameter (mm) incubated on V-8 juce agar for 72 hours in the dark at 25°C.

[2]Nine isolates of *Pythium* and 15 isolates of *Mortierella* were compared.

[3]ANOVA indicated significant differences between means (F = 440.517; P=0.0005).

Disease Management

This investigation indicated that soil adjacent to irrigation lines, which remained in place during fumigation, contained higher populations of *Pythium* spp., at least during the second growing season following fumigation. These higher populations resulted in increased root infection of seedlings growing nearer irrigation lines; such infection probably caused seelings to grow more slowly than less infected seedlings growing further from irrigation lines. *Pythium* spp. often colonize and destroy feeder roots, causing reduced intake of nutrients and moisture by infected seedlings (Vaartaja and Bumbieris 1964). Problems may be exacerbated because soils near irrigation structures tend to receive more moisture during the growing season. Wetter soils would be more conducive to proliferation and spread of *Pythium* spp. since their motile spores have flagella which aid movement through water-saturated soils (Lange and Olson 1983).

Pythium spp. tend to occur at potentially damaging levels in many portions of the Coeur d'Alene Nursery despite repeated soil fumigation, whereas levels of *Fusarium* spp. are usually kept low (James and Gilligan 1986b; James and others 1990). Proportionately fewer propagules per g of soil are required for *Pythium* spp. to elicit conifer seedling disease than for *Fusarium* spp. (James and others 1990; Vaartaja and Bumbieris 1964). For example, 100 cfu/g of *Pythium* is usually the threshold where disease may be expected, whereas the threshold for *Fusarium* is closer to 1000 cfu/g. In most cases, *Fusarium* populations remain below this threshold at the Coeur d'Alene Nursery, whereas *Pythium* levels may occasionally exceed their threshold. However, noticeable disease is often lacking in soils with relatively high *Pythium* levels at the nursery, although sublethal growth effects may occur.

Removing irrigation structures prior to fumigation to ensure uniform coverage of fumigant will probably reduce "edge" effects on seedlings growing next to these structures. However, it is also important to reduce soil saturation near irrigation lines when possible to control buildup and spread of *Pythium* spp. Spot treatments with metalaxyl (Subdue®) may be necessary if seedlings display disease symptoms (mortality or chlorosis and stunting) in poorly drained portions of fields. By ensuring uniform application of soil fumigants, providing proper soil drainage, and properly controlling irrigation, future effects of *Pythium* root disease may be minimized at the Coeur d'Alene Nursery.

ACKNOWLEDGEMENTS

We appreciate assitance of Lionel Hall with field data collection and Dr. G. Fowler (University of Michigan - Ann Arbor) with data analysis. We also appreciate the manuscript review provided by R. K. Dumroese (University of Idaho).

LITERATURE CITED

Armstrong, G. M. and J. K. Armstrong. 1975. Reflections on the wilt fusaria. Ann. Rev. Phytopathol. 13:95-103.

Bloomberg, W. J. 1973. Fusarium root rot of Douglas-fir seedlings. Phytopathology 63:337-341.

Bloomberg, W. J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology 66:1090-1092.

Booth, C. 1966. The genus *Cylindrocarpon*. Mycological Papers No. 104. Commonweath Mycological Institute, Kew, Surrey, UK. 56p.

Campbell, S. J. and B. R. Kelpsas. 1988. Comparison of three soil fumigants in a bareroot conifer nursery. Tree Planters' Notes 39(4):16-22.

Domsch, K. H., W. Gams and T.-H. Anderson. 1980. Compendium of soil fungi. Academic Press, London. 859p.

Edmonds, R. L and W. A. Heather. 1973. Root diseases in pine nurseries in the Australian Capital Territory. Plant Dis. Reptr. 57:1058-1062.

Fisher, N. L., L. W. Burgess, T. A. Toussoun and P. E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium*. Phytopathology 72:151-153.

Hayes, A. J. 1965. Some microfungi from Scots pine litter. Trans. Brit. Mycol. Soc. 51:293-309.

Hendrix, F. F., Jr. and W. A. Campbell. 1968. Pythiaceous fungi isolated from southern forest nursery soils and their pathogenicity to pine seedlings. Forest Science 14:292-297.

Iannelli, D., R. Capparelli, G. Cristinzio, F. Marziano, F. Scala and C. Noviello. 1982. Serological differentiation among formae speciales and physiological races of *Fusarium oxysporum*. Mycologia 74(2):313-319.

James, R. L. 1982. Pythium root disease of Douglas-fir and grand fir seedlings at the Coeur d'Alene Nursery, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 82-10. 10p.

James, R. L. 1987. Chlorosis of Engelmann spruce transplant seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Nursery Disease Notes No. 64. 3p.

James, R. L. 1988. Diseases of conifer seedlings associated with *Cylindrocarpon* species: a review. USDA Forest Service, Northern Region. Forest Pest Management. Nursery Disease Notes No. 76. 14p.

James, R. L. 1991. Colonization of Engelmann spruce and lodgepole pine seed with *Mortierella* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Nursery Disease Notes No. 115. 7p.

James, R. L., R. K. Dumroese, C. J. Gilligan and D. L. Wenny. 1989. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Idaho Forest, Wildlife and Range Exp. Sta. Bull. No. 52. 10p.

James, R. L. and C. J. Gilligan. 1984. Studies of *Fusarium* associated with containerized conifer seedling diseases: pathogenicity tests of isolates from the Alpine Nursery, Kalispell, Montana. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 84-14. 29p.

James, R. L. and C. J. Gilligan. 1986a. Root diseases of western white pine transplants at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 86-11. 8p.

James, R. L. and C. J. Gilligan. 1986b. Soil populations of *Fusarium* and *Pythium* in Field 10, USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Nursery Disease Notes No. 32. 3p.

James, R. L. and C. J. Gilligan. 1988a. Association of *Fusarium* with nondiseased containerized ponderosa pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 88-5. 10p.

James, R. L. and C. J. Gilligan. 1988b. Occurrence of *Fusarium* on the roots of nondiseased bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 88-12. 4p.

James, R. L. and C. J. Gilligan. 1990. Root decay of container-grown western white pine seedlings - Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region. Rept. 90-10. 18p.

James, R. L. and P. B. Hamm. 1985. Chlamydospore-producing species of *Phoma* from conifer seedlings in Pacific Northwest forest tree nurseries. Proc. Montana Acad. Sci. 45:26-36.

James, R. L., S. Metzger and C. J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Forest Pest Management. Rept. 90-11. 18p.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.

Kuninaga, S. and R. Yokosawa. 1989. Genetic relatedness within and between formae speciales of *Fusarium oxysporum* as measured by DNA-DNA reassociation kinetics. Ann. Phytopathol. Soc. Japan. 55:216-223.

Lange, L. and L. W. Olson. 1983. The fungal zoospore - its structure and biological significance. *In*: S. T. Buczacki (ed.). Zoosporic Plant Pathogens - A Modern Perspecitve. Academic Press, London. pp. 1-44.

Matturi, S. T. and H. Stenton. 1964. Distribution and status in the soil of *Cylindrocarpon* species. Trans. Brit. Mycol. Soc. 47:577-587.

Matuo, T. and O. Chiba. 1966. Species and formae speciales of Fusaria causing damping-off and root-rot of coniferous seedlings in Japan. Ann. Phytopathol. Soc. Japan. 32:14-22.

Middleton, J. T. 1943. The taxonomy, host range, and geographic distribution of the genus *Pythium*. Memoirs of the Torrey Botanical Club. 20:1-171.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. *Fusarium* species. An illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Nirenberg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Can. J. Bot. 59:1599-1609.

Park, D. 1959. Some aspects of the biology of *Fusarium oxysporum* Schl. in soil. Ann. Bot. 23:35-49.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Ann. Rev. Phytopathol. 23:23-54.

Price, D. 1984. *Fusarium* and plant pathology: the reservoir of infection. *In*: Moss, M. D. and J. E. Smith (eds.). The Applied Mycology of *Fusarium*. Cambridge University Press, Cambridge, UK. pp. 71-93.

Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Sewell, G. W. F. and J. C. Brown. 1959. Ecology of *Mucor ramannianus* Moeller. Nature 183:1344-1345.

Tint, H. 1945. Studies in the Fusarium damping-off of conifers. III. Relation of temperature and sunlight to the pathogenicity of *Fusarium*. Phytopathology 35:498-510.

Vaartaja, O. 1967. Damping-off pathogens in South Australian nurseries. Phytopathology 57:765-768.

Vaartaja, O. and M. Bumbieris. 1964. Abundance of *Pythium* species in nursery soils in South Australia. Aust. J. Bio. Sci. 17:436-445.

Waterhouse, G. M. 1968. The genus *Pythium*. Mycological Papers No. 110. Commonwealth Mycological Institute, Kew, Surrey, UK. 71p.